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The Regiochemistry and Relative Reaction Rates of Methylbiphenyl Isomers in Electrophilic Aromatic Substitution Reaction (EAS) Nitrations Suggest a Non-Planar Geometry for 2-Methylbiphenyl while 3- and 4-Methylbiphenyl Remain Planar

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THE REGIOCHEMISTRY AND REALATIVE REACTION RATES OF METHYLBIPHENYL ISOMERS IN ELECTROPHILIC AROMATIC SUBSTITUTION REACTION (EAS) NITRATIONS SUGGEST A NON-PLANAR GEOMETRY FOR 2-METHYLBIPHENYL WHILE 3- and 4-METHYLBIPHENYL REMAIN PLANAR

by

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ABSTRACT

Electrophilic aromatic substitution (EAS) reactions have long been a fundamental addition to sophomore-level organic chemistry classes, allowing students the opportunity to explore the electron donating and withdrawing effects of electrons contained in the substituents of the aromatic reactant. In this paper we present preliminary findings on the nitration of methylated biphenyls using kinetic and regioselective assessments to analyze steric influences on the planarization of 2-methylbiphenyl after EAS nitration. Our preliminary findings show that nitration favors the methylated phenyl ring of 2-methylbiphenyl, indicating that the steric influence of the methyl group restricts planarization of the carbocation intermediate. Furthermore, a competition nitration reaction between biphenyl and toluene provides proof of concept for kinetic assessment of nitration rates that will eventually be applied to 2-methylbiphenyl; this competitive nitration showed that biphenyl nitrates 1.87 ± 0.61 (95% C.I.) times faster than toluene.

Liberal Arts Connection:

Victor

The results presented here and the methods used to draw accurate conclusions from our data provide unique insight into how we, as a human society, develop frameworks for understanding what is true; additionally, it provides an assessment of techniques used to teach others about these perceived truths. In the field of chemistry, it is difficult to use the five human senses to directly observe changes in chemical systems; instead, we must indirectly observe changes through instrumental analysis, color changes, analytical techniques, or by using established literature and theories to extrapolate conclusions based on observations of experiments. We cannot use our senses to directly observe if 2-methylbiphenyl adopts a coplanar conformation when nitrated; instead, we had to create a model based on established theories (sigma and pi donating and withdrawing effects) to extrapolate what the nitration products would look like if 2-methylbiphenyl is able to planarize or not. Alternatively, we can use molecules known to be similar to 2-methylbiphenyl—toluene, biphenyl, 3-methylbiphenyl, and 4-methylbiphenyl—to assess how 2-methylbiphenyl reacts differently relative to these models and extrapolate reasons for these differences using established theories. This is not to say that we can directly observe where the nitrate group goes on 2-methylbiphenyl or the kinetics of relative nitration rates between 2-methylbiphenyl and toluene; instead, we must rely upon instrumental analysis—such as retention times on chromatography columns—and analytical calibration techniques—such as internal and external standard linear curves—in order to assess the data and conclude the truth about what is happening in our observed chemical system.

Tristan

While this project may seem to focus solely on the molecular minutia of the 2-methylbiphenyl isomer encapsulating our focus, any work of science reaches far beyond the purely scientific. The moment we begin interpreting what we see or what our instruments tell us, the lens that we view the world through frames what we see. This fact, while unavoidable, is also crucial. The training that I have received in chemistry frames the way that I view data, approach problem solving, and draw conclusions. Without this training, experience in the field, and reading papers, I would not have been able carry out this project like I did. Likewise, the general public is made up of countless individuals who have their own lenses through which they view the world, attuned to their experience and what is expected of them.

For better or for worse, this means that the vast public community that receives scientific communication will not view that information the same way a scientist would. This is compounded by the fact that scientific discoveries are often relayed to the public as findings unaccompanied by data. Without data, conclusions cannot be measured against the evidence. Novel scientific findings are often viewed as

absolute truth when aspects like the accuracy of instruments, the number of trials, and quality of reagents are never seen. Thus, a discrepancy exists between the way a scientist views truth and a member of the public views truth. While this may not be a big deal for research like ours, where our audience is fellow organic chemists who will take our conclusions alongside the data, many scientific findings affect the lives of the public. Recently the dissemination of information on COVID-19 is an example of science distributed through the media that can have significant effects on the lives of the readers.

These are just a few of the ways our scientific work is punctured by the humanities. Whenever interpretation or communication enters into the equation, the work done by scientists moves beyond simple science.

The Regiochemistry and Relative Reaction Rates of Methylbiphenyl Isomers in Electrophilic Aromatic Substitution Reaction (EAS) Nitrations Suggest a Non-planar Geometry for 2-methylbiphenyl, while 3 and 4-methylbiphenyl Remain Planar.

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Abstract:

Electrophilic aromatic substitution (EAS) reactions have long been a fundamental addition to sophomore-level organic chemistry classes, allowing students the opportunity to explore the electron donating and withdrawing effects of electrons contained in the substituents of the aromatic reactant. In this paper we present preliminary findings on the nitration of methylated biphenyls using kinetic and regioselective assessments to analyze steric influences on the planarization of 2-methylbiphenyl after EAS nitration. Our preliminary findings show that nitration favors the methylated phenyl ring of 2-methylbiphenyl, indicating that the steric influence of the methyl group restricts planarization of the carbocation intermediate. Furthermore, a competition nitration reaction between biphenyl and toluene provides proof of concept for kinetic assessment of nitration rates that will eventually be applied to 2-methylbiphenyl; this competitive nitration showed that biphenyl nitrates 1.87 ± 0.61 (95% C.I.) times faster than toluene.

Keywords: Nitration, methylbiphenyl, electrophilic aromatic substitution, kinetics, regiochemistry, relative reaction rates, transition state geometries

Introduction/background

Electrophilic aromatic substitution (EAS) reactions are a fundamental class of reactions explored in sophomore-level organic chemistry courses[1]. In this class of reactions, different substituents on the benzene ring of the reagent cause the reaction to favor different regiochemistry in the products and to react at different rates. These substituents can be divided into two main categories: activating groups and deactivating groups. Activating groups *donate* electron density to the aromatic ring and result in *-ortho* and *-para* products and facilitate faster reaction rates[2]. Deactivating groups *withdraw* electron density from the ring, resulting in *-meta* products that form at a slower rate[2].

To determine whether a substituent is activating or deactivating, two methods of electron density donation and withdrawal must be considered. The first is the σ effect, named after the sigma bond between the ring and the substituent. Electronegativity of the substituent atom is the main factor considered—if the substituent has a higher electronegativity than the sp^2 carbon it is bound to, then it will be σ-withdrawing. The π effect, on the other hand, is caused when the pure p orbitals on the aromatic sp^2 carbon overlap with the p orbitals of the substituent. These two effects can work together to cause a substituent to be activating or withdrawing or oppose one another.

Phenol, for example, has opposing σ and π effects. Oxygen's high electronegativity compared to the aromatic sp^2 carbon makes it σ-withdrawing, but its lone pair pure p orbital overlap with those of the carbon making it π-donating. Experimental evidence shows that the -OH substituent is activating, as phenol reacts more quickly than

benzene and directs the reaction to the -*ortho* and -*para* positions[1]. This allows the conclusion to be drawn that the π-donating effect of the -OH substituent in phenol outweighs the σ-withdrawing effect.

The presence of pure p orbitals on the substituent alone, however, does not guarantee that the π effect will occur. Biphenyl, for example experiences π-donation when it is planar, as the p-orbital on the two connecting sp^2 hybridized carbons can overlap. However, if the two rings are instead at a 90° dihedral angle, the pure p-orbital cannot overlap, resulting in no π-donation and no activation. If the two rings of biphenyl are not coplanar, and the π-donation cannot occur, the only effect is the σ-withdrawing effect of the other benzene ring. If this effect is sufficiently electron withdrawing, a non-coplanar phenyl substituent could instead act as a deactivating, meta-directing substituent. Because biphenyl gives EAS reaction products that are ortho and para and happen quickly, it can be concluded that the π-donation is significant, and the geometry of the two rings is planar.

In this project we investigate the geometry of 2-methylbiphenyl, which may not favor the planar positioning (See A vs. A' in **Figure 1**). The methyl group adjacent to the aromatic substituent provides steric hinderance that works against the two rings becoming planar. While the barrier to becoming planar for biphenyl is less than 2 kcal/mol,[3] the barrier for 2-methylbiphenyl is larger than 10 kcal/mol, at least 5 times higher[4]. If an EAS reaction proceeds through a planar transition structure, then this barrier will result in a higher activation energy. Our goal is to investigate the planarity of the rings of 2-methylbiphenyl by observing the regiochemistry of the EAS reaction products along with its rate compared to those of similar molecules.

The major product of nitration of the two other methylbiphenyl isomers is known[5], occurring ortho and para to the methyl group for 3-methylbiphenyl (B in **Figure 1**) and para to the ring with the methyl group for 4-methylbiphenyl (**C in Figure 1**). The observed major products of nitration of 3- and 4-methylbiphenyl can be reasoned out with straightforward resonance arguments if it is assumed that the two rings are able to adopt a co-planar geometry, like biphenyl. Based on similar arguments and assuming the two rings can adopt a coplanar geometry, 2-methylbiphenyl would nitrate at the same location as 4-methylbiphenyl, as that position allows for the positive carbocation of the intermediate to resonate through the rings and on the methyl group (A in **Figure 1**). However, should the ring assume a non-planar geometry, the π-donation cannot occur, and so the only effect is the σ-withdrawing effect of the other benzene ring. This means that the primary nitration location would be para to the methyl group (as the activating substituent) and meta to the benzene substituent (as the withdrawing substituent). While it could nitrate ortho to the methyl, this position would not be favored because of the steric hindrance of the methyl group.

The reluctance of 2-methylbiphenyl to planarize after electrophilic attack can also be assessed using a kinetic model; by comparing the rate of nitration on 2-methylbiphenyl relative to biphenyl and toluene, the influence on the steric hindrance of the methyl group on planarization can be qualitatively assessed. If the methyl group allows for planarization of the carbocation intermediate, the nitration rate will be quicker for 2-methylbiphenyl relative to toluene and biphenyl because the aromatic rings can π-donate to delocalize the positive charge; this effect is stronger than the orthro-para directing effect than the σ-donation of the methyl group of toluene, even with slight σ-withdrawing from the aromatic ring. However, if the methyl group prevents planarization in 2-methylbiphenyl after EAS, the two rings are unable to π-donate; this makes nitration favored on the methylated ring of 2-methylbiphenyl due to the σ-donating effect to the methyl group, though the nitration rate will be slower than toluene because toluene possesses one extra σ-donating hydrogen which is replaced by a phenyl constituent in 2-methylbiphenyl that neither σ-donates nor π-donates. Likewise, 2-

methylbiphenyl will also nitrate slower than 3- and 4-methylbiphenyl and may react more slowly than biphenyl itself due to deactivation by the rotated phenyl group in 2-methylbiphenyl—which is activating in biphenyl—competing against the activating methyl group in methylbiphenyl isomers. We show that relative nitration rates are quantifiable by using a preliminary competitive nitration reaction model of biphenyl and toluene nitrated in a deficit of nitric acid. This model allows us to conclude that biphenyl and toluene are both viable for competitive nitration with 2-methylbiphenyl.

In addition, gas chromatography mass spectroscopy (GC-MS) was utilized for qualitative molecular identity assessment and quantification of biphenyl and toluene instead of NMR quantification for multiple reasons, the primary being that our lab lacks access to an NMR with high enough resolution for quantification and the research was mostly conducted during the SARS-CoV-2 2019-2021 pandemic, preventing access to high resolution NMR at neighboring labs. GC-MS qualitative assessment of compounds was also favored due to lower limits of detection than NMR for minor products of reactions, relative ease of use, and ability to identify a multitude of constituents in solution. Quantitative GC-MS was favored due to unavailability of NMR quantification and ease of ability to prepare multiple calibration points for multipoint linear curve fitting.

When completed, this research will uncover deeper insight into the understanding of how benzene and methyl substituents and their regiochemistry affect the products of EAS reactions. Nitration is a crucial reaction that allows the creation of a 'foothold' on a benzene ring, which can then be transformed into other functional groups through a plethora of other synthetic reactions[1]. Documenting major products, reactant rates, and resulting conditions in this project adds to the body of organic synthetic knowledge. These discoveries could thus help inform the synthetic pathways that medicinal chemists and process chemists develop to synthesize potential drug candidates.

Materials and Methods:

Synthesizing and purifying the 2-methylbiphenyl isomer (A). The 2-methylbiphenyl isomer and other methylbiphenyl isomers were synthesized by Suzuki-Miyaura coupling[6,7] phenylboronic acid and 2-bromotoluene. Several different procedures were attempted to purify the resulting 2-methylbiphenyl, including recrystallization in water/ethanol systems, spinning band column distillation, preparatory GC, and isopropanol recrystallization at -80° C.

Finding nitration conditions. Nitration was attempted with biphenyl and toluene to find ideal conditions for synthetic and competitive nitration. Nitric acid and sodium nitrate were tested as nitrate sources at varying stoichiometric amounts, temperatures, and solvent. Synthetic mononitration conditions were found to be most effective with 2:3 molar ratio of biphenyl or toluene to sodium nitrate with acetic anhydride as a solvent, sulfuric acid as a catalyst, and room temperature to mild heating. Competitive nitration conditions were found to be most effective with 2:1:0.8 molar ratio of toluene to biphenyl to nitric acid with 17.45 M acetic acid as a solvent, sulfuric acid as a catalyst, and hot water heating.

Overview of regiochemistry investigation. After 2-methylbiphenyl was subjected to the optimized synthetic mononitration conditions, a GC-MS of the results was taken. To identify the regiochemistry of the nitration reaction, a comparison with isolated isomers of known connectivity were needed. Attempts were made to synthesize and purify the different nitrated 2-methylbiphenyl products. This was first done by subjecting 2-bromotoluene to nitration and attempting to isolate individual isomers. Because the isomers could not be isolated, a Suzuki-Miyaura coupling with a mixture of the three isomers was attempted. A different synthetic

method was applied later, where toluene (**D**) was nitrated at the para position (**E**), brominated adjacent to the methyl group (**F**), and finally coupled to phenylboronic acid (**G**) through Suzuki-Miyaura coupling. This yielded **H** (2-methyl-5-nitro-1,1'-biphenyl). The regiochemistry of one of the original nitration products was then determined by comparing the retention times and mass spectra of the nitration products with the synthesized product.

Calibration curve preparation. Calibration curves for toluene and biphenyl were prepared by massing a standard of either toluene (Fischer Science) or biphenyl (99%, Acros Organics) in a tared volumetric flask and diluting to the mark with methanol (99.8%, Alfo Aesar). Volumetric glassware was then used to prepare several dilutions of this standard with a consistent concentration of ethylbenzene (99%, Aldrich) for use as an internal standard. Three GC-MS samples were taken for each solution and each sample was ran three times. Microsoft Excel Version 2103 was then used to create linear fits for toluene and biphenyl using TIC Multipoint External Standard Method, TIC Multipoint Internal Standard Method, Single Ion External Standard Method, and Single Ion Internal Standard Method using ethylbenzene as an internal standard[8].

Overview of kinetic investigation. A competition nitration reaction between toluene and biphenyl was used to assess the relative reaction rates of toluene and biphenyl for EAS nitration and test the applicability of kinetic analysis for 2-methylbiphenyl Toluene (Fischer Science) and biphenyl (99%, Acros Organics) were massed in the same round bottom flask and dissolved in 17.45 M acetic acid. 15.8 M nitric acid was massed in the same round bottom and then 18 M sulfuric acid was added, and the reaction flask warmed in hot water for 107 minutes. The reaction mixture was quenched with saturated sodium bicarbonate and extracted with 22.3 mL diethyl ether (99.8%, Fischer Chemical). The ether was diluted with methanol (99.8%, Alfa Aesar) to 100 mL with an ethylbenzene (99%, Aldrich) internal standard and biphenyl and toluene contents analyzed with quantitative GC-MS and Single Ion External Standard Method.

RESULTS

Suzuki-Miyaura Coupling and attempted purification. All the Suzuki-Miyaura coupling reactions we carried out between phenylboronic acid and 2-bromotoluene had the biphenyl dimer as a byproduct in addition to unreacted 2-bromotoluene and phenylboronic acid (confirmed by GC-MS in **Figure 3**). Of the purification methods attempted both recrystallization in water/ethanol systems and spinning band column distillation failed to isolate 2-methylbiphenyl. Preparatory GC purified out the remaining reactants but was unable to separate out the biphenyl from the mixture (confirmed by GC-MS in **Figure 4**). The preparatory GC method also damaged the column beyond repair. This was the best purification we completed, and so our nitration reaction with 2-methylbiphenyl also had traces of biphenyl in it (~5%). A -80° recrystallization with varying amounts of isopropanol was also attempted. Crystallization did occur, and supernatant liquid could be removed from the solid. However, the success of this strategy at removing biphenyl from 2-methylbiphenyl is still being determined.

Nitration of 2-methylbiphenyl. The 2-methylbiphenyl with 5% biphenyl was nitrated under the optimized synthetic mononitration conditions, yielding the GC-MS spectrum **Figure 5** using the MeBP-2 method (see supplemental information). In the range of expected products, four peaks can be seen that have an MI of 213. The first peak is at 10.892 min (**a** in **Figure 5**) and contains both an M-17 peak and an M-28 peak. The second peak is at 11.751 min (**b** in **Figure 5**), and it contains an M-17 peak. The third and fourth peaks are clustered around 13 min, with one at 12.957 min (**c** in **Figure 5**) and one at 13.034 min (**d** in **Figure 5**). Neither of these peaks contain an M-17 or M-28 peak.

Nitration of 2-bromotoluene and subsequence coupling. After nitrating 2-bromotoluene, we were unable to separate the isomers from each other (GC-MS in **Figure 6**). The mix was then subjected to Suzuki-Miyaura coupling with phenylboronic acid. However, very few products formed. After several trials enough nitrated product formed to be visible in the predicted region (GC-MS in **Figure 7**).

Synthesizing H (2-methyl-5-nitro-1,1'-biphenyl). Nitrating toluene resulted in a mix of ortho and para isomers, and multiple recrystallizations in hexanes at -20° C followed by vacuum filtration gave pure p-nitrotoluene (confirmed by GC-MS in **Figure 8**). After bromination and several ethanol recrystallizations, the pure 2-bromo-4-nitrotoluene (confirmed by GC-MS in **Figure 9**) was coupled to phenylboronic acid in a Suzuki-Miyaura coupling (GC-MS in **Figure 10**).

Kinetic assessment of nitration using several calibration methods. Molecular quantity in moles for both biphenyl and toluene was assessed after seven nitration reactions (see supplemental information) using TIC Multipoint External Standard Linear Curve, TIC Multipoint Internal Standard Linear Curve, Single Ion External Standard Linear Curves, and Single Ion Internal Standard Linear Curves using ethylbenzene as an internal standard. The initial molecular quantity of each reagent was assessed through massing the reagent before reaction, multiplying by manufacturer assessment of percent purity, and dividing by molecular mass. Assuming negligible dinitration of reagents, the sum of the differences in initial and unreacted toluene and biphenyl is equal to the molar quantity of nitrate consumed as shown in Equation 1.

$$nitrate_0 = biphenyl_0 - biphenyl_\infty + toluene_0 - toluene_\infty$$

Equation 1

Subtracting the moles of consumed nitrate from the moles of nitrate initially added to the reaction generates an assessment of moles of unreacted nitrate. Table 1 tabulates the molar quantities of toluene, biphenyl, and nitrate for the competition reaction of 0.1339 g biphenyl (0.8596 mmol) (99%, Acros Organics) and 0.2438 g toluene (2.646 mmol) with 0.894 mmol nitrate from concentrated nitric acid.

Surprisingly, the calibration methods that did not utilize the internal standard ethylbenzene converged on similar molar quantity assessments for both biphenyl and toluene, marked with asterisks in Table 1. Due to the failure of all three multipoint internal standard linear curves to predict a molar value with greater consistently than multipoint external standard method, a single ion 154 multipoint external calibration curve (Equation 2) was used to assess molar quantity of unreacted biphenyl and a single ion 91 multipoint external calibration curve (Equation 3) was used to assess molar quantity of unreacted toluene.

$$Abundance\text{-}_{154} = 6.95E9 * [biphenyl] + 3.00E5\ M$$

Equation 2

$$Abundance\text{-}_{91} = 2.13E9 * [toluene] - 3.04E4\ M$$

Equation 3

The relative rate of reaction for the nitration of two aromatic substances was shown by Ingold and Shaw to obey Equation 4 for any concentration of nitrate where the nitrate is in molar deficit relative to the aromatics:

$$\frac{k_{biphenyl}}{k_{toluene}} = \frac{\log\left(1-\left(\frac{nitrate_0}{biphenyl_0}*\frac{R}{1+R}\right)\right)}{\log\left(1-\left(\frac{nitrate_0}{toluene_0}*\frac{1}{1+R}\right)\right)}$$

Equation 4

Where $k_{biphenyl}/k_{toluene}$ is the relative rate of reaction, $nitrate_0$ is the moles of nitrate consumed by the nitration of toluene and biphenyl determined using Equation 1, $biphenyl_0$ and $toluene_0$ are the initial moles of biphenyl and toluene respectively before nitration, and R is the molecular ratio of biphenyl to toluene defined as $R = (biphenyl_0 - biphenyl_\infty)/(toluene_0 - toluene_\infty)$ where $biphenyl_\infty$ and $toluene_\infty$ are the remaining molar quantities of biphenyl and toluene respectively after nitration.

By inserting the molar quantities determined with single ion multipoint external standard calibration curves (Table 1, lightly shaded values) into Equation 4 the rate of nitration for biphenyl relative to toluene, $k_{biphenyl}/k_{toluene}$ was determined to be 1.87± 0.048 (standard error). As this assessment used single replicate analysis (t value 12.706 for 95% confidence)[8] the precision of this preliminary value is low, with a 95% confidence interval of ±0.61. Future kinetic assessments, particularly of 2-methylbiphenyl, will utilize multiple replicates for quantification of competitive nitration.

DISCUSSION

Regiochemical analysis of nitration sites on 2-methylbiphenyl:

Four peaks were seen in the GC-MS when 2-methylbiphenyl was nitrated at our optimized conditions (**Figure 5**). The identity of peak **d** at 13.034 min was confirmed to be 2-methyl-5-nitro-1,1'-biphenyl (**H**), as its retention time and mass spectrum matches those of the synthesized 2-methyl-5-nitro-1,1'-biphenyl (**H**) in **Figure 10**. Qualitative TIC analysis predicts that this peak is the most abundant of the products of mononitration of 2-methylbiphenyl, suggesting that 2-methylbiphenyl assumes the non-planar geometry of **A'** in **Figure 1**.

The identities of the other three peaks in **Figure 5** can be extrapolated from literature MS spectra, but more work is needed to confirm the identities. The literature MS spectra of 2-bromo-6-nitrotoluene and 2-nitrotoluene both show that a fragmentation of M-17 is present when a nitro group is adjacent to a methyl group on a benzene ring. This is theorized to be caused by one of the oxygens on the nitro group leaving with one of the hydrogens on the adjacent methyl group. This M-17 is also present when the nitro group is adjacent to a phenyl substituent, like in *o*-nitrobiphenyl. However, when adjacent to the benzene substituent, the MS also shows an M-28 peak. This is assumed to be an oxygen bonded to a carbon atom, though we are unsure as to how or why this fragmentation actually occurs.

However, we can use these pieces of information to make an educated guess as to the identities of **a**, **b**, and **c** in **Figure 5**. Because the MS of **a** shows both an M-17 and an M-28 peak, we believe that the nitro group is adjacent to a phenyl substituent. While this could be on either ring, the non-planar geometry suggested by the identity of **d** means that the ring with the methyl group will be the more activating of the two, and so we hypothesize that **a** is 2-methyl-6-nitro-1,1'-biphenyl (**a**). The MS of **b**, on the other hand shows only the M-17 peak, suggesting that the nitro group is adjacent to the methyl group. As such, we suspect that **b** is 2-methyl-3-nitro-1,1'-biphenyl. Finally, **c** has neither the M-17 or the M-28 peak, which suggests that the nitro group is not adjacent to any other substituents. Because we know that **d** is 2-methyl-5-nitro-1,1'-biphenyl, this leads us to conclude that **c** is 2-methyl-4-nitro-1,1'-biphenyl. Similar to the argument for **a**, this *could be* the 2-methyl-4'-nitro-1,1'-biphenyl product of **A** in **Figure 1**, the ring with the methyl group will have more activation than the other ring as stated above.

The placement of **c** on the ring with the methyl group is further confirmed by **Figure 7**, which shows the crude products of the nitration of 2-bromotoluene coupled to phenylboronic acid. The one small peak that resulted has a retention time of 12.966 minutes, matching the retention time of **c** at 12.957 minutes. Because the nitro group was on the bromotoluene and not the phenylboronic acid, this suggests that the nitration of **c** occurred on the ring with the methyl group.

Kinetic analysis of competitive nitration rates between toluene and biphenyl:

Successful completion of a nitration competition reaction for toluene and biphenyl took seven nitration attempts (see supplemental) and will serve as a basis for conducting competitive nitration of toluene and 2-methybiphenyl, as well as biphenyl with 2-methylbiphenyl. The conclusion that biphenyl nitrates 1.87 ± 0.61 times more quickly than toluene is consistent with predictions of σ and π donating differences between the two compounds[1,2,9]. Six of the seven

competitive nitration reactions attempted were deemed unsuccessful due to either 1) disappearance of toluene that could not be explained by reaction with the amount of nitrate present (attempts 1-3), 2) lack of nitrated products in GCMS TIC chromatograms (attempts 5 and 6), or 3) calibration generating a molar quantity of toluene after the competition reaction that is larger than the molar amount of toluene initially added to the reaction (attempt 4). It was important to first establish successful competitive nitration conditions before using 2-methylbiphenyl in competition reactions as 2-metylbiphenyl is difficult and time consuming to synthesize and purify; given that 2-methylbiphenyl is structurally similar to biphenyl and toluene, it is reasonable to extrapolate that nitration conditions that yielded quantifiable biphenyl and toluene will also yield quantifiable 2-methylbiphenyl.

The reason for the failure of multipoint internal standard method to assess biphenyl and toluene molar abundance after competition reactions is unknown, though assessment of potential causes is warranted. The fourth, fifth, sixth, and seventh competition reactions were performed simultaneously on different stir plates and used the same solution of ethylbenzene as an internal standard. The inconsistency of detector response for ethylbenzene was assessed to be negligible (>3% relative standard deviation) for competitions that had homogenous sample dilution in volumetric flasks shown in Table 2. The failure of the multipoint internal standard to assess reagent molar quantities after competition reactions appears to not be related to the ability of the detector to repeatably assess ethylbenzene detector response.

However, consistency in methods utilized for correlation of ethylbenzene detector response to concentration was found to be erroneous. This is seen when comparing the ratio of the average detector response to ethylbenzene over concentration of ethylbenzene assessed as seen in Table 3. The linearity of the external standard calibration curves produced provides a useful fallback for quantitative analysis though their reliability is questionable. This is because slight changes in the flow rate of the chromatography column, variability in split ejection, and as well as other determinate instrumental errors may affect detector response variably from one run to the next [8]. This determinacy was mitigated in the creation of the calibration curves by generating calibration points in replicate, though assessment of the biphenyl and toluene molar presences after nitration was not assessed in replicate due to time constraints before preliminary report drafting. This reduces our confidence in the quantitative value for the difference in nitration kinetics for biphenyl and toluene but does not decrease our confidence that the conditions for competitive nitration would be applicable for future competition reactions between 2-methylbiphenyl and biphenyl or toluene.

Future Work:

Purifying 2-methylbiphenyl and synthesizing the other nitrated 2-methylbiphenyl isomers:

For future work, pure 2-methylbiphenyl needs to be isolated. Preparatory GC could be used to isolate the 2-methylbiphenyl with traces of biphenyl in the future and the mass percentage of the biphenyl contaminate could be assessed with the calibration curves we have constructed. However, it would be best to simply come up with an effective method of purification as we cannot assess kinetic conditions for 2-methylbiphenyl in competition with any aromatic—besides biphenyl—as long as the biphenyl contaminate is present. We believe that the -80° C recrystallization has the potential to be the solution to this problem. More trials are needed to investigate the viability of the approach. Working on a quicker transfer of the solution off the precipitate could solve the issue of ineffective purification. The solvent and solvent ratios used for extraction of impurities from the crystalized product could also be changed to troubleshoot this issue.

The synthesis of 2-methyl-5-nitro-1,1'-biphenyl confirmed the regiochemistry of the most abundant product in the nitration reaction. However, synthesis of the other nitrated 2-methylbiphenyl isomers is necessary to confirm the other

mononitration products (**a**, **b**, and **c** in **Figure 5**). In particular, o-nitrotoluene could be purified from our nitrotoluene syntheses and brominated. While two main isomers could form from the bromination, it must be tried to see if one can be isolated. In addition, to prepare for a potential publication, NMR spectra need to be taken of all the isolated and purified compounds to confirm their identities after the conclusion of the SARS-CoV-2 pandemic.

One proposed synthetic route is mononitration of bromobenzene to form p-nitrobromobenzene. In addition, 2-methyl-phenylboronic acid could by synthesized by reacting 2-bromotoluene with $B(OCH_3)_3$ in a Grignard reaction[1]. The p-nitrobromobenzene could then be coupled to the 2-methyl-phenylboronic acid to form the nitration product where the nitro group is para to the ring with the methyl group. As this is the product we expect would result if 2-methylbiphenyl were planar, this could be used to potentially identify one of the peaks on the 2-methylbiphenyl nitration (GC-MS reference).

The ortho isomer of the toluene nitration could also be isolated through the methods mentioned in our p-nitrotoluene purification source[10]. The o-nitrotoluene could then be brominated by our methods[11,12], yielding 6-bromo-2-nitrotoluene and 4-bromo-2-nitrotoluene. If one of these could be isolated, then it could be coupled to phenylboronic acid and isolated to yield another of the nitrated methylbiphenyl isomers.

Finishing calibration curves and carrying out the full suite of competition reactions:

After >1.4 mmol of 2-methylbiphenyl has been isolated and purified, we plan to prepare calibration curves using the same procedure outlined in the supplemental information for toluene calibration curve preparation with the exception of massing the ethylbenzene dropwise instead of pouring it into the volumetric flask. Alternatively, a different internal standard may be selected due to difficulty correlating ethylbenzene concentration from detector response (see supplemental information). Ideally, the calibration curves for biphenyl and toluene would be reassessed using ethylbenzene massed dropwise or by adding an alternative internal standard; however, due to the time and material constraints this reassessment would require and the reproducibility and precision of the current multipoint external calibration curves, this is does not appear to be a necessity and is not recommended.

Following the creation of calibration curves for 2-methylbiphenyl the relative nitration reactivity of 2-methylbiphenyl and biphenyl as well as 2-methylbiphenyl and toluene should be assessed by adding two aromatics to concentrated nitric acid in concentrated acetic acid and sulfuric acid in a stoppered flask submerged in a hot water both. These reaction conditions were determined to be effective for biphenyl and toluene competitive nitration and will hopefully mitigate the need to use any 2-methylbiphenyl to determine competitive nitration conditions.

We are fortunate to have first-year Honors student Alessandro Rizzi continuing our work over the Summer and next year. We wish him the best of luck as he continues onward with our research.

EXPERIMENTAL

Methylbiphenyl isomers (A, B, and C in Figure 1). 7 mmol of phenylboronic acid was dissolved into 25 mL of ethanol in a 125 mL Erlenmeyer flask. 5 mmol of 2-bromotoluene was then added, followed by 2.0 mL of $PdCl_2$ in 5% HCl. After waiting two minutes, 10 mL of KOH was added. After 60 minutes 25 mL of deionized water was added to quench remaining base, and two 10 mL extractions with methylene chloride were performed. The combined organic layers were washed twice with 10mL deionized water, dried over sodium sulfate, and removed under vacuum to yield a brown oil. This procedure was carried out with 3-bromotoluene and 4-bromotoluene to synthesize 3-methylbiphenyl and 4-methylbiphenyl. This was the basic recipe used throughout the project for the methylbiphenyl isomers, scaled when needed. **Figure 3** shows the resulting GC-MS from one of these reactions. In addition to the desired product 2-methylbiphenyl

(**c**), the dimer biphenyl (**b**) was created. Residual unreacted 2-bromotoluene also remained (**a**), in addition to unreacted phenylboronic acid, which manifested as a phenylboronic acid trimer.

Purifying 2-methylbiphenyl with preparatory GC. Crude 2-methylbiphenyl oil was injected in 200 uL or 600 uL aliquots onto a non-polar stationary phase GOW-MAC® Gas Chromatography Thermal Conductivity Detector using a helium mobile phase, bridge current of 81 mA, injection port temperature of 250 degrees Celsius, detector temperature of 209 degrees Celsius, Column Temperature of 169 degrees Celsius, outlet temperature of 202 degrees Celsius, and flow rate of 60 mL/min. Sample elution was monitored using the thermal conductivity detector on LoggerPro. Ethanol solvent had a retention time of 50 seconds, 2-bromotoluene at 90 seconds, and product at 4.8 minutes. A glass u-tube was manually created by heating and bending a glass tube into the shape of a "U" with a perpendicular bend at the top that fit the GOW-MAC outlet. Product was collected for one minute by placing the u-tube over the GOW-MAC outlet at least three minutes after injection after the appearance of vapor and while submerging the bottom of the u-tube in water. 2-methylbiphenyl, phenylboronic acid, and biphenyl condensed near the outlet and 2-methylbiphenyl and biphenyl condensed at the bottom of the u-tube. The 2-methylbiphenyl and biphenyl from the bottom of the u-tube was kept as semi-pure product (see GC-MS in **Figure 4**).

Nitration of 2-methylbiphenyl. 0.1986 g of the crude 2-methylbiphenyl with biphenyl contaminate isolated from the bottom of the u-tube from preparative gas chromatography was massed in a pointed small vial. 3.0 mL of acetic anhydride was added with a graduated plastic pipet and the solution was stirred with a triangular stir vein. 0.1438 g of sodium nitrate was massed in a weigh boat and added to the vial, yielding no color change. 0.25 mL of 18 M sulfuric acid was added to the vial and solution turned orange and evolved heat. The reaction was allowed to proceed with no additional heating at room temperature for 3 hours. A few drops of the solution were worked up with saturated sodium bicarbonate and extracted in ether. This resulted in the GC-MS in **Figure 5**.

Attempted purification of 2-methylbiphenyl with -80° recrystallization. Differing amounts of isopropanol (0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL, and 10 mL solvent, respectively) were pipetted into cone-cap vials with 0.5 mL of crude 2-methylbiphenyl product from the Suzuki-Miyaura coupling. A vial with just 0.5 mL product along with a vial with 1.5 mL of isopropanol were included to see if either froze on its own. The vials were then placed into the -80° C freezer.

Nitration of 2-bromotoluene. 0.9615 g (5.62 mmol) of 2-bromotoluene was massed into a 20 mL beaker with a stir bar, followed by 5.6 mL of acetic anhydride and 0.6038 g (7.0 mmol) of sodium nitrate. 0.6 mL of 18 M sulfuric acid was added, turning the solution hot and yellow. After 55 min, the mixture was poured over 30 mL of ice water in a 50 mL beaker, forming yellow oil and some solid at the bottom. After three extractions with 20 mL of ether, the organic layers were combined, washed four times with 20 mL sodium bicarbonate, and dried over sodium sulfate overnight. Because the GC-MS showed significant acetic acid and acetic anhydride peaks, the ether layer was washed three more times with 20 mL sodium bicarbonate, dried again over sodium sulfate, and removed under vacuum to yield a yellow-orange oil (see GC-MS in **Figure 6**).

SM Coupling of crude nitrated 2-bromotoluene. 0.5495 g of crude nitrated 2-bromotoluene product was weighed into a 125 mL Erlenmeyer flask with 10 mL of ethanol and 0.3034 g (2.49 mmol) of phenylboronic acid. 1 mL of $PdCl_2$ in 5% HCl was added, and after two minutes, 10 mL of 1 M KOH was added. 18 hours later the solution was a dark drown orange color. Several Pasteur pipettes full of the reaction mixture were taken into a sep funnel with 15 mL of ether. After shaking, some of the ether was put into a GC-MS vial with sodium sulfate (see GC-MS in **Figure 7**).

Synthesizing E (4-nitrotoluene)[4]. To make a 40 mL solution of 20 mL 65% HNO_3 and 20 mL 98% H_2SO_4, 1.75 mL of deionized water was added

to a 50 mL beaker, followed by slow addition of 18.25 mL of 16 M HNO_3 and 20 mL of 98% H_2SO_4. The addition happened on ice and the solution remained on ice until used. 20 g (0.217 mol) of toluene was added to a 125 mL Erlenmeyer flask and placed in a 7° C ice bath. The 40 mL of chilled acid solution was then added slowly over 20 minutes, and the solution was kept between 6° and 10° C. Once the addition was complete, the solution was allowed to come to 14° C, where it was maintained between 13.5° and 14.5° C for two hours. It was then placed in a room temperature water bath for an hour, then poured over 300 mL of iced water in a 1000 mL beaker and left overnight. The following morning (18 hours later) three 50 mL ether extractions were performed, combined, and washed three times with 50 mL of saturated sodium bicarbonate. The first wash was orange and the second two were yellow. The organic layer was washed two further times with 50 mL water, dried over sodium sulfate, and removed under vacuum to yield 15 mL yellow liquid (see GC-MS in **Figure 8**).

Purifying E (p-nitrotoluene)[5]. The resulting yellow liquid was placed in the -20° C freezer. 24 hours later, the supernatant was pipetted off the crystals, and 6 mL of hexanes were added. This was repeated 4-5 times, and the final iteration was vacuum filtered and rinsed with chilled hexanes, yielding 1.2910 g of pure p-nitrotoluene (**GC-MS spectra?**)

Synthesizing F (2-bromo-4-nitrotoluene[11,12]. 4 mL of 18 M H_2SO_4 was added dropwise to 4 mL of deionized water on ice to make 50:50 ratio by volume H_2SO_4. A 50 mL RBF was covered in tin foil to prevent light entering, and 1.0157 g of p-nitrotoluene was added, followed by the 50:50 ratio by volume H_2SO_4 (which was first allowed to come to room temperature). After stirring with a stir bar for 10 minutes, 1.3034 g of n-bromosuccinimide were added. After 24 hours, three ether extractions of 10 mL were performed. The organic layers were combined, washed twice with 20 mL saturated NaCl, dried over sodium sulfate, then removed under vacuum to yield ~1 g of crude product. The GC-MS revealed a successful bromination. Two successive recrystallizations in ethanol and a vacuum filtration yielded 0.6063 g of purified 2-bromo-4-nitrotoluene (see GC-MS in **Figure 9**).

Synthesizing H (2-methyl-5-nitro-1,1'-biphenyl). To a 10 mL RBF, 0.2497 g (1.1558 mmol) of 2-bromo-4-nitrotoluene was added, followed by 6 mL of ethanol, 0.1793 g (1.471 mmol) of phenylboronic acid, and 0.5 mL of $PdCl_2$ in 5% HCl. After stirring for 2 min, 2.5 mL of 1 M KOH was added. 15 min after the start of the reaction, some of the reaction mixture was pipetted into a sep funnel with 5 mL deionized water and 7 mL of ether. After shaking the ether layer was used to make a GC-MS (**TW-4-12-15a**). 18 hours later, the reaction mixture was poured into a separation funnel with 15 mL of deionized water, and 2.5 extractions with 10 mL dichloromethane were carried out. The organic layers were combined, washed 2 times with 25 mL of deionized water, dried over magnesium sulfate, and removed under vacuum to yield a dark brown liquid (see GC-MS in **Figure 10**).

Competitive nitration reaction. 0.1339 g biphenyl (0.8596 mmol) (99%, Acros Organics) and 0.2438 g toluene (2.646 mmol) (Fisher Science) were massed in a tared 25 mL round bottom flask. 17.45 M acetic acid was added until dissolution of biphenyl without stirring to produce a clear colorless solution. The flask was tared, and 0.0905 g concentrated nitric acid (15.8 M) was added dropwise followed by 0.5 mL of concentrated sulfuric acid (18 M) and two stir triangles and a stir bar. The flask was stoppered and clamped shut to prevent evaporation and placed in hot water (initial temperature 41.8 degrees Celsius, final temperature 74.6 degrees Celsius) with stirring for 107 minutes, creating a dark black-brown solution. Contents of the flask (including stirring apparatuses) were transferred slowly to a separation funnel containing saturated sodium bicarbonate and diethyl ether, then the 25 mL flask contents were rinsed into the funnel with sodium bicarbonate and ether three times; the total volume of saturated bicarbonate used was 17.1 mL and diethyl ether 22.3 mL. The separation funnel was then inverted with shaking and venting three

times, creating a top clear orange phase and a bottom clear yellow phase. The top phase was drained into a 100 mL flask containing 10.00 mL of ethylbenzene solution and diluted to below the mark with 99.8% methanol rinsed through the separation funnel; the volumetric flask was inverted 5 times, generating pressure that was released by uncapping the flask and precipitated a white salt. After the solution settled, additional methanol was added to fill the flask to the mark and inverted 5 additional times, generating additional pressure with was released again by uncapping the solution after the fifth inversion. GCMS of this vial was taken directly and analyzed without further dilution. Ethylbenzene solution was transferred from a 100 mL volumetric flask of 0.4826 g ethylbenzene (4.500 mmol) (99%, Aldrich) diluted to the mark with 99.8% methanol with a 10.00 mL volumetric pipet. Multipoint external calibration curves for toluene and biphenyl were used to assess the remaining concentration of toluene and biphenyl after nitration. Detector response was measured for toluene with single ion 91.00 mass per charge and single ion 154.00 mass per charge for biphenyl.

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We would like to thank Seattle Pacific University for providing the facilities, equipment, and reagents we used throughout this project. We would also like to thank Dr. Karisa Pierce for consultation regarding our analytical methods and figure preparation. We would also like to thank Sue Martin and Kristi Holt, who have let us use their lab spaces, equipment, and chemicals throughout this project in addition to being available for chemical consultation and ordering needed reagents. Finally, we would like to thank Josh Padilla and Alessandro Rizzi for their invaluable assistance in carrying out procedures for the project.

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Figures and Tables

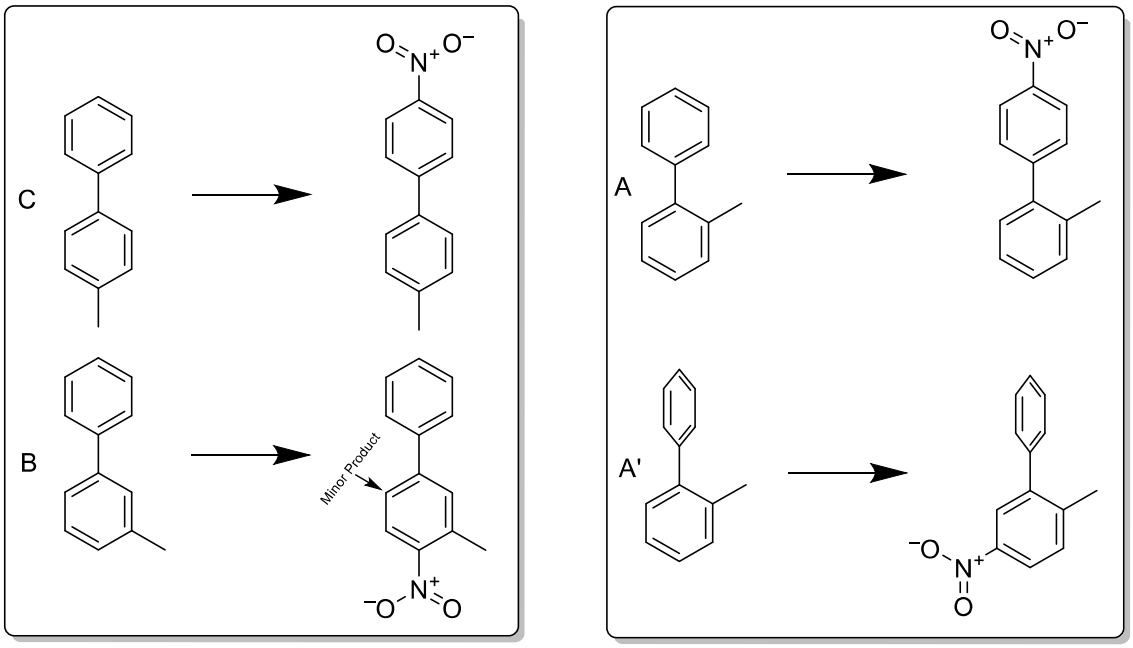

Figure 1. Known nitration positions for 3-methylbiphenyl (B) and 4-methylbiphenyl (C). In addition, the two different nitration sites predicted by the planar (A) and a 90° dihedral angle (A') geometries of 2-methylbiphenyl.

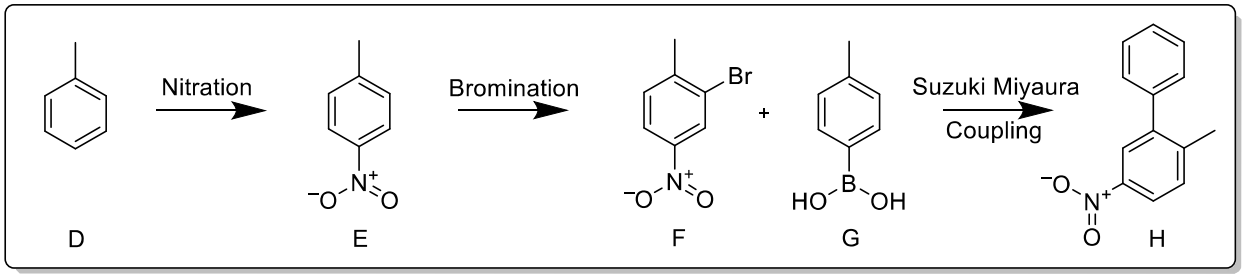

*Figure 2. The synthetic scheme to creating 2-methyl-5-nitro-1,1'-biphenyl (**H**)*

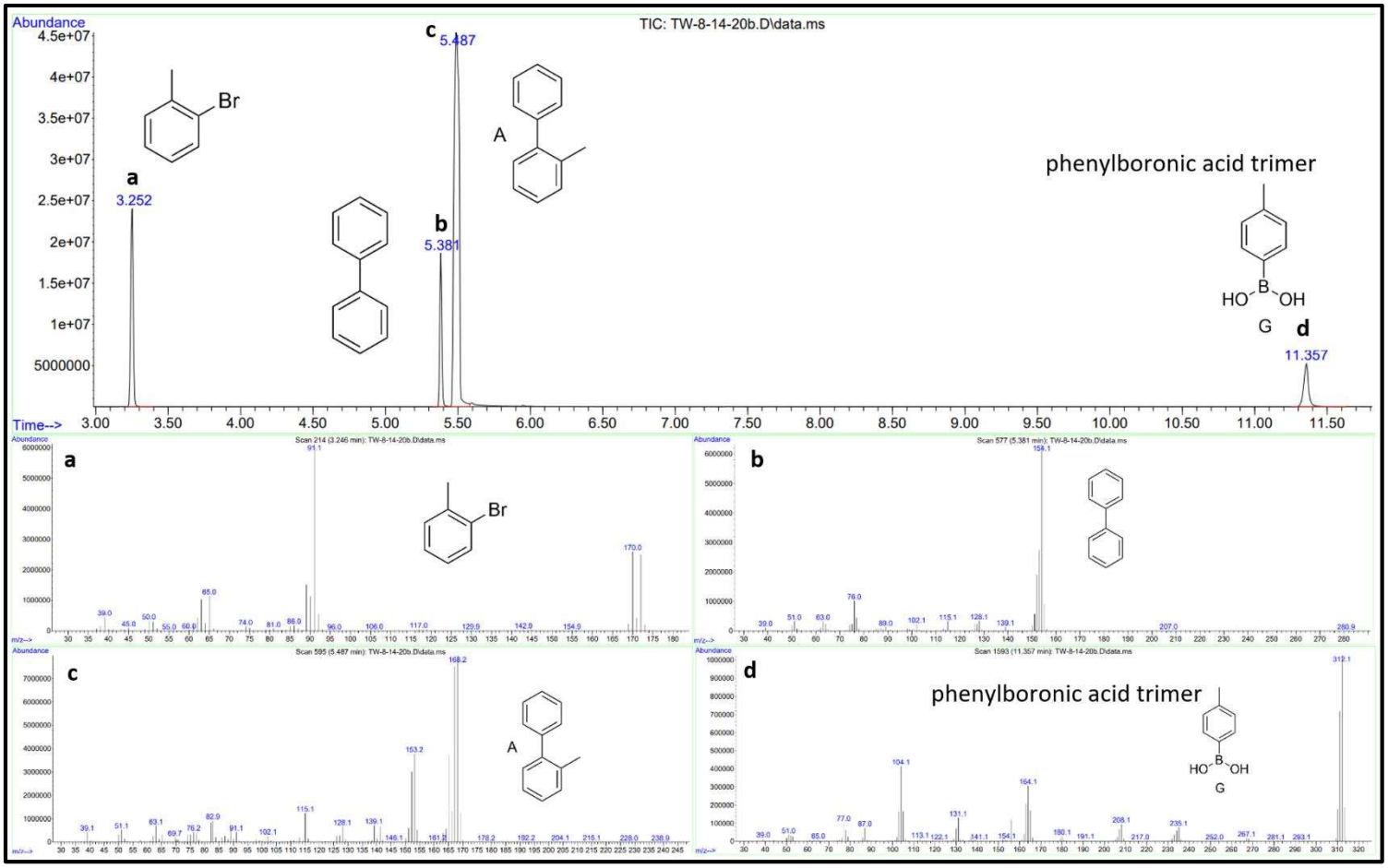

*Figure 3. The TIC and MS's of the original synthesis of 2-methylbiphenyl through a Suzuki-Miyaura coupling of 2-bromotoluene and phenylboronic acid. In addition to the desired product 2-methylbiphenyl (**c**), the dimer biphenyl (**b**) was created. Residual unreacted 2-bromotoluene also remained (**a**), in addition to unreacted phenylboronic acid, which manifested as a phenylboronic acid trimer. The method was MeBP,* and the molecules were identified through the GC-MS library.

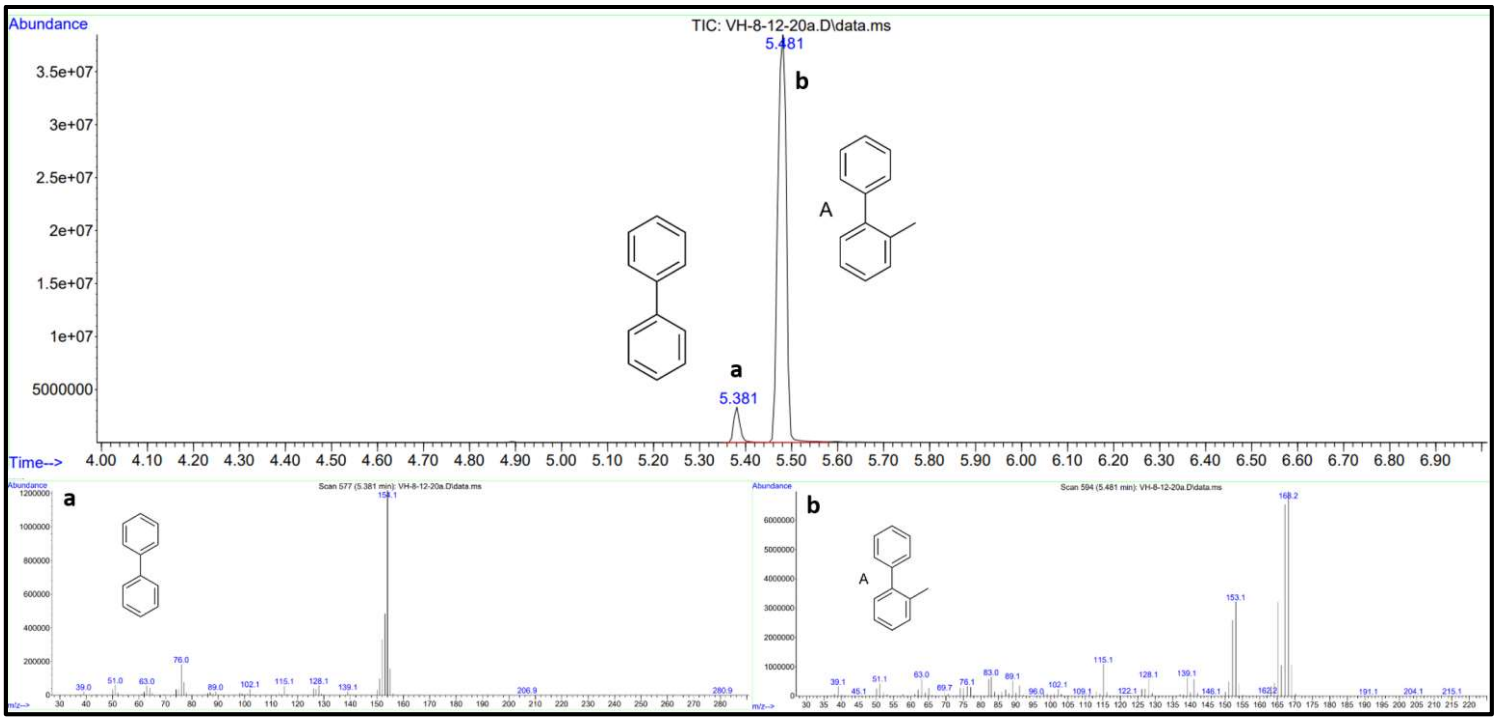

*Figure 4. The Suzuki-Miyaura coupling products from Figure 3 purified using preparatory GC. The biphenyl peak (**a**) is significantly smaller, and there is no more unreacted 2-bromotoluene or phenylboronic acid, only 2-methylbiphenyl (**b**) and biphenyl (**a**). The method was MeBP,* and the molecules were identified through the GC-MS library.

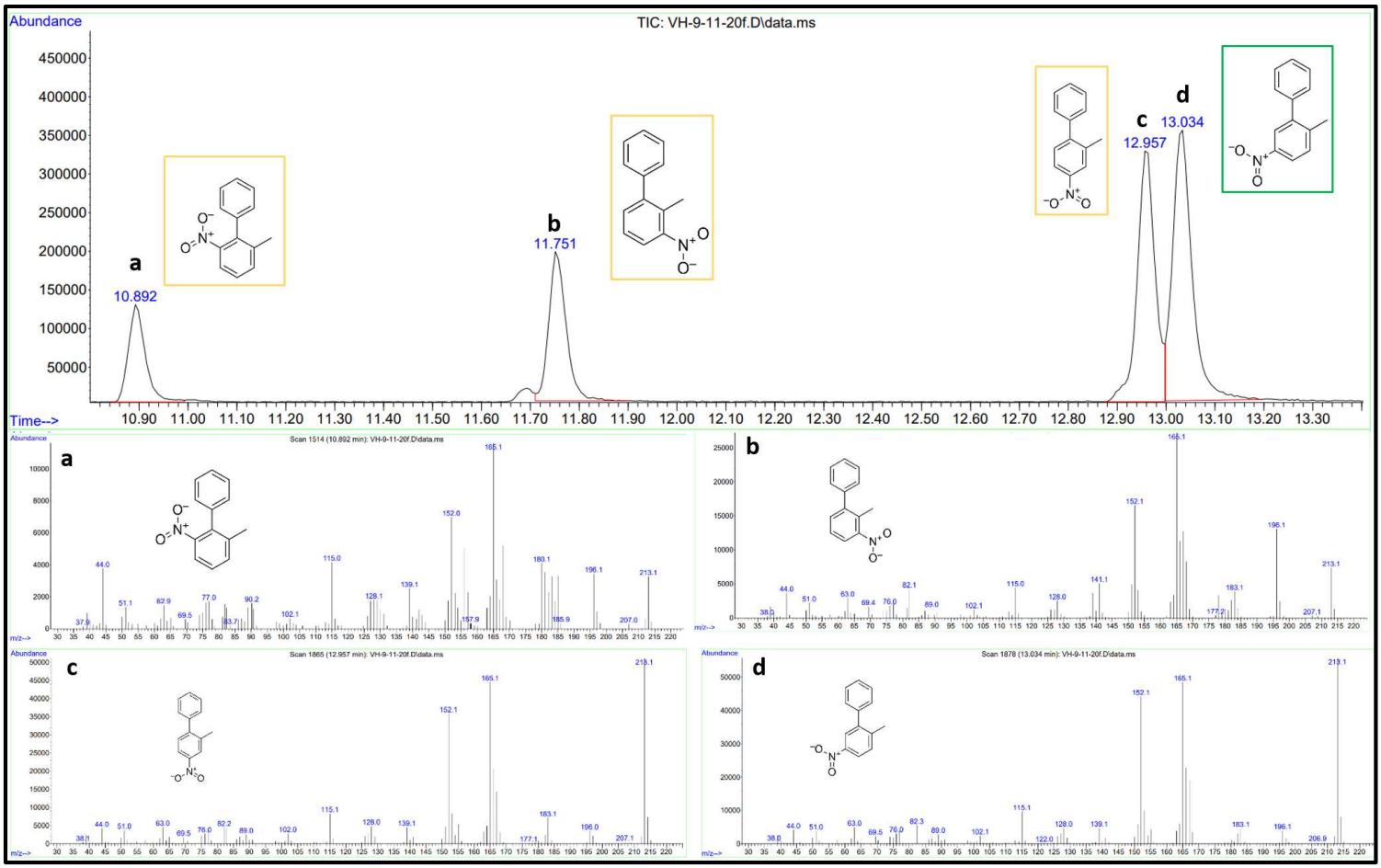

*Figure 5. The nitration of prep-GC purified 2-methylbiphenyl, zoomed in on the retention times with MI's at 213. In yellow (**a, b,** and **c**) are our educated guesses at the identity of the molecules based on fragmentation arguments. In green is the molecule we confirmed by comparing its retention times to the synthesized 2-methyl-5-nitro-1,1'-biphenyl (**H**) in **Figure 10**. The method was MeBP-2.*

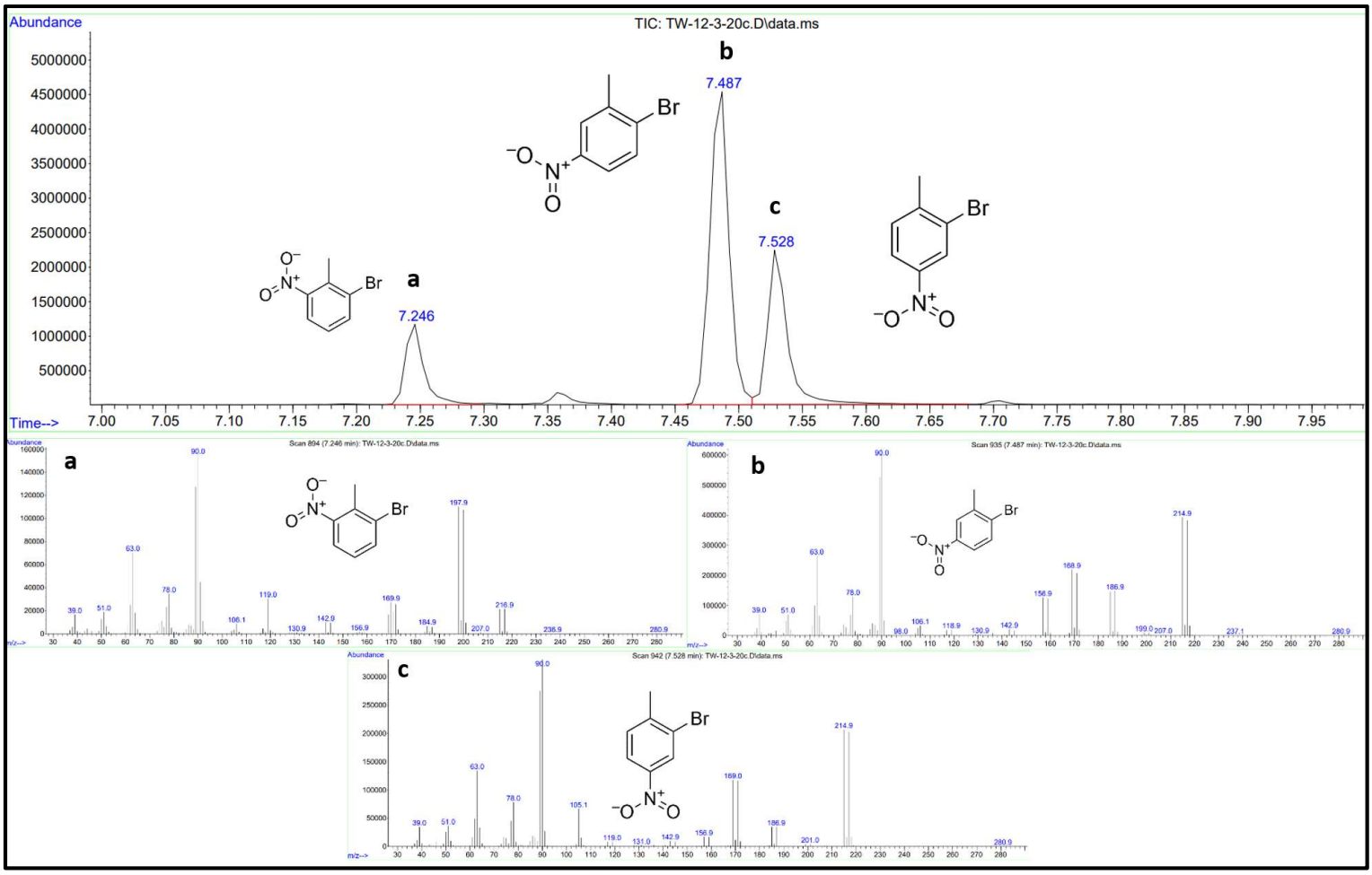

*Figure 6. Products from the nitration of 2-bromotoluene, which yielded three isomers **a, b,** and **c**. The method was NiTol, and the molecules were identified through the GC-MS library.*

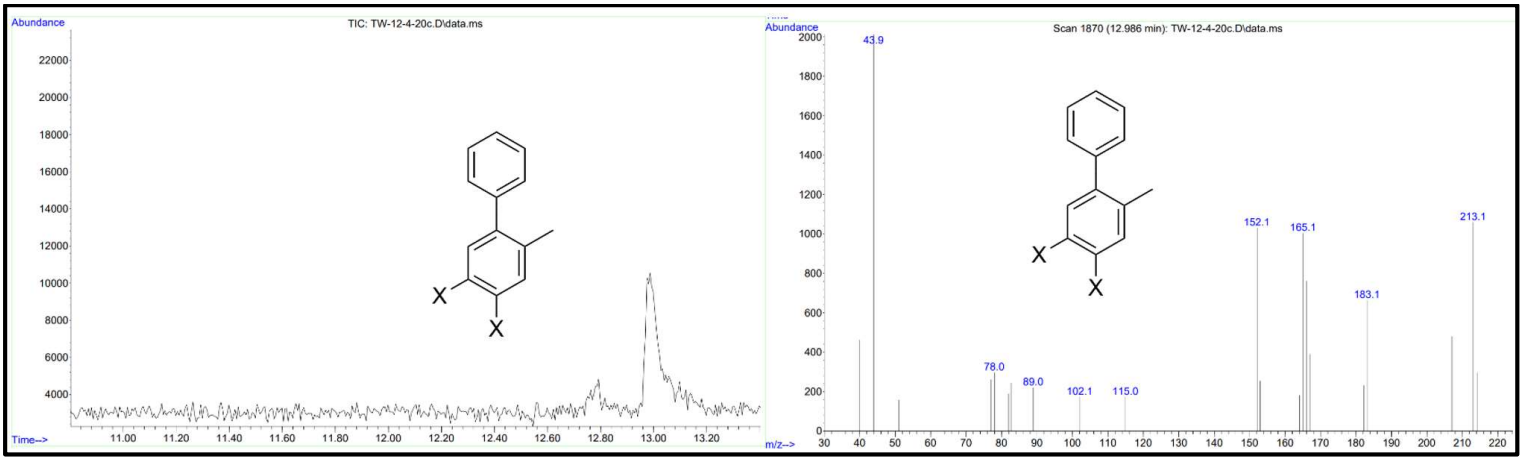

*Figure 7. Results from Suzuki-Miyaura coupling the crude products from **Figure 6** with phenylboronic acid. Based on fragmentation arguments, we excluded the isomer where the nitro group is adjacent to the methyl group. The method was MeBP-2 and can thus be directly compared to **Figure 5**.*

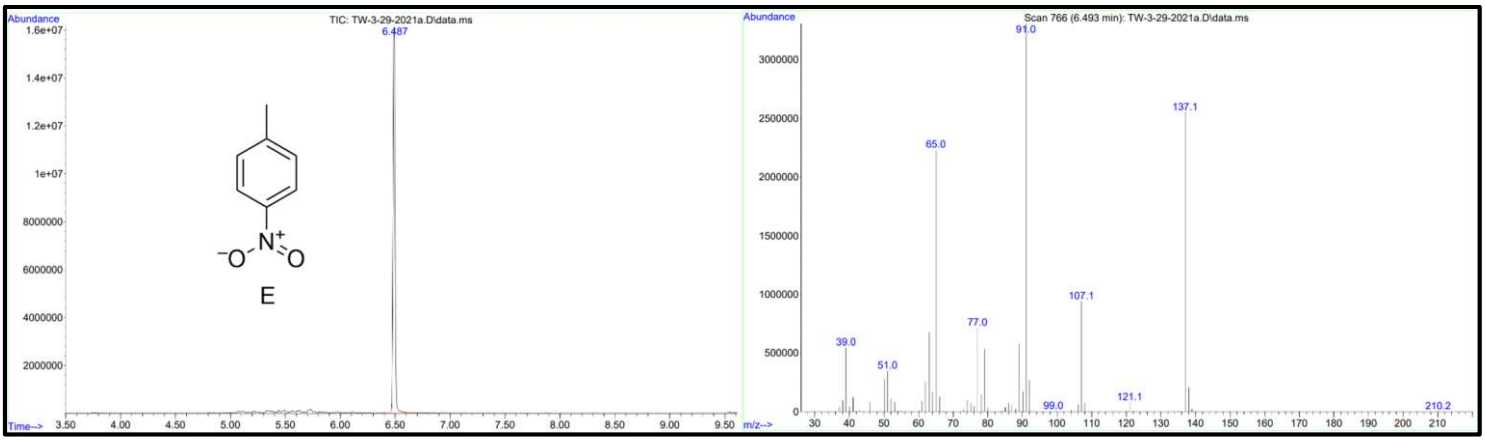

*Figure 8. Recrystallized p-nitrotoluene (**E**) from the nitration of toluene. The method was NiTol-2,* and the molecule was identified through the GC-MS library.

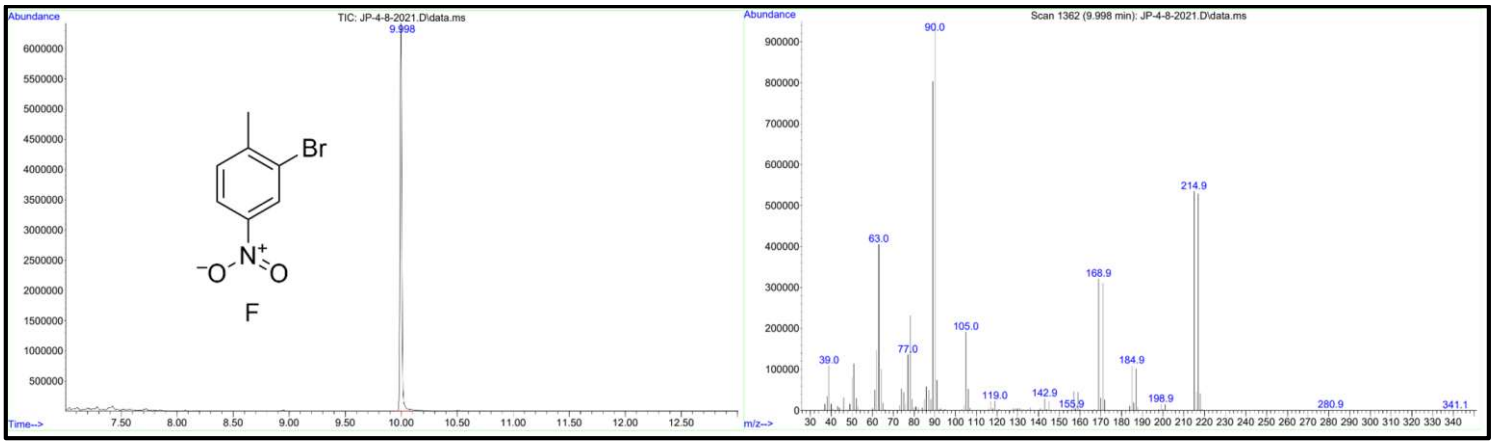

*Figure 9. Recrystallized 2-bromo-4-nitrotoluene (**F**) after brominating p-nitrotoluene (**E, Figure 8**). The method was ORGANIC,* and the molecule was identified through the GC-MS library.

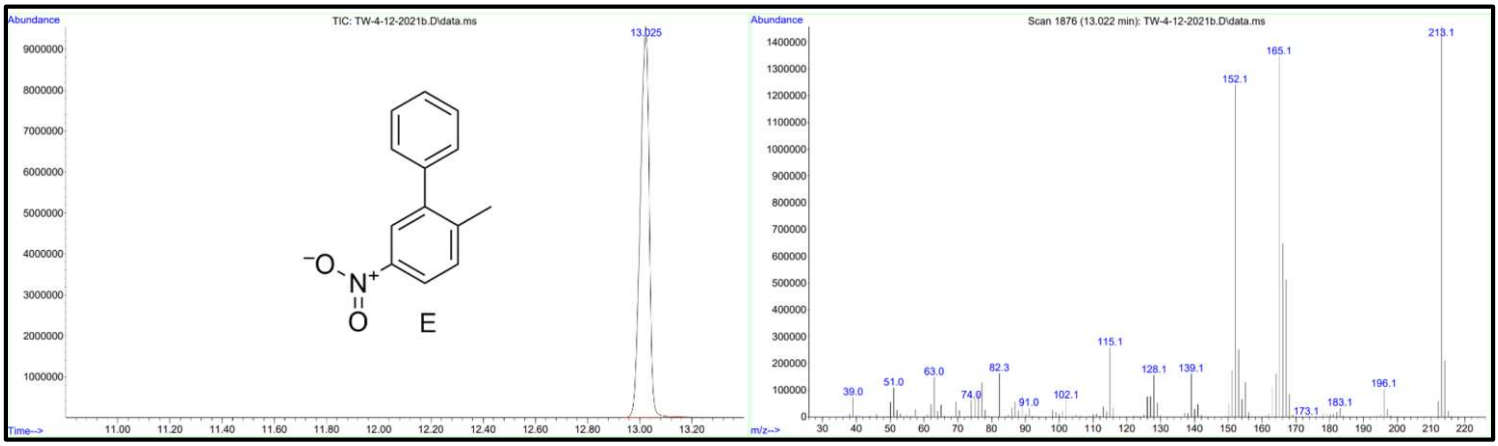

*Figure 10. Recrystallized 2-methyl-5-nitro-1,1'-biphenyl (**E**) after Suzuki-Miyaura coupling 2-bromo-4-nitrotoluene (**F, Figure 9**) with phenylboronic acid (**G**). The method was MeBP-2, and the molecule was identified by knowing the reactants and matching the MW of the anticipated product to that of the actual product.*

Calibration Method Utilized	Unreacted Moles of Toluene	Initial Moles of Toluene	Unreacted Moles of Biphenyl	Initial Moles of Biphenyl	Moles of Nitrate Consumed	Moles of Nitrate Unconsumed
TIC External Standard	0.002396*	0.002646	0.00064100 *	0.0008162	0.00042541	0.00058156
TIC Internal Standard	0.0004515	0.002646	0.00000000	0.0008162	0.00301063	-0.0020037
SI External Standard, SI 91 for Toluene	0.002431*	0.002646	0.00068680 *α	0.0008162	0.00034451	0.00066246
SI External Standard, SI 65 for Toluene	0.002389*	0.002646		0.0008162	0.00038599	0.00062098
SI Internal Standard, SI 91 for Toluene	0.0008101	0.002646	0.00000000 α	0.0008162	0.00265210	-0.0016451
SI Internal Standard, SI 65 for Toluene	0.003292	0.002646		0.0008162	0.00017008	0.00083689

Table 1. Assessment of molar quantities of reagents after reaction in hot nitric acid (1.01 mmol) for 107 minutes. Multipoint internal standard calibration assessments shows inconsistent assessment of reagent presence after reaction contrasted to consistent assessment of reagents with multipoint external standard calibration, marked with an asterisk. This trend is reflected in other nitration reaction attempts (see supplemental information).*

α SI 154 used to assess moles of biphenyl for both quantifications.

Competition Reaction Attempt	Total Ion Count for Ethylbenzene	SI 106 for Ethylbenzene
Four	51839956	7181431
Five	50085838	6948636
Six (top phase)	65578842	9457568
Six (bottom phase)	39874025	5562506
Seven	49919775	6997181
Standard Deviation Excluding Reaction Attempt Six	±1063924	±122813
Standard Deviation for All Four Attempts	±10567518	±1613576

Table 2: Assessment of scatter in detector response for internal standard ethylbenzene for four competition reactions that utilized the same solution of internal standard at the same dilution. The lack of homogeneity in the volumetric flask when diluting the reaction contents from the sixth competition reaction (see supplemental information) explains the inconsistent detector response for this competition reaction attempt.

Assessments that Utilized Ethylbenzene as an Internal Standard	Concentration of Ethylbenzene Analyzed by GC-MS (M)	Average Detector Response Ethylbenzene TIC	Ratio of Average Detector Response Over Concentration (M^{-1})
Biphenyl Calibration Curves	0.002568	22505913	8763525666
Toluene Calibration Curves	0.003775	15420620	4084932405
Competition Reactions 4-5, 7	0.004500	50615190	11247290228

Table 3: Assessment of concentration of ethylbenzene analyzed and average detector response for ethylbenzene for three aspects of the experimental method that used ethylbenzene as an internal standard. Biphenyl calibration, toluene calibration, and competition reactions 4, 5, and 7 used the same source of 99% ethylbenzene but utilized different massed aliquots of ethylbenzene. All biphenyl calibration samples used the same 0.1377 g aliquot of ethylbenzene. All toluene calibration samples used the same 0.1002 g aliquot of ethylbenzene. Competition reactions 4, 5, and 7 used the same 0.4826 g aliquot of ethylbenzene.

SUPPLEMENTAL MATERIALS

Supplemental Methods

GC-MS methods:

An Agilent Technologies 7890B GC System with an Agilent J&W DB-5 nonpolar polysiloxane GC column and helium mobile phase attached to an Agilent Technologies 5977B MSD mass spectrometer was used for quantitative and qualitative GCMS. Qualitative GCMS chromatograms of solid or liquid samples were assessed by diluting less than 100 mg of the analyte sample with acetone or ether in a 2 mL GCMS vial with a rubber septum and injected onto the column using an Agilent Technologies 7693

Autosampler. Quantitative GCMS chromatograms for calibration curve preparation and competition reaction assessment were prepared in 100 mL volumetric flasks diluted to the mark with methanol; vials were filled with the methanol solution and injected onto the column in replicate using the autosampler. Quantitative and qualitative samples were run with either the NiTol-2 method using an initial oven temperature of 50 C for 2 minutes, 30 C/min ramp to 150 C, 5 C/min ramp to 200 C, followed by a 30 C/min ramp to 250 C for a total tun time of 22 minutes, the NiTol method using an initial oven temperature of 50 C for 2 minutes, 30 C/min ramp to 250 C, then hold at 250 C for 5 minutes for a total tun time of 13.7 minutes, or the MeBP-2 method using an initial oven temperature of 100 C for 2 minutes, 30 C/min ramp to 150 C, 5 C/min ramp to 200 C, followed by a 30 C/min ramp to 250 C for a total tun time of 15.3 minutes. Automated integration was used to determine peak areas for quantification on Chem Station Enhanced Data Analysis using an initial threshold of 13.0, initial area reject of 0, initial peak width of 0.080, and shoulder detection OFF. Qualitative sample identity was assessed using Chem Station and NIST 2011 library.

Biphenyl Calibration Curve Preparation:

0.4301 g biphenyl (99%, Acros Organics) was massed in a 100 mL volumetric flask and diluted to the mark with 99.8% methanol to create solution **1A**. 0.1377 g of 99% ethylbenzene was massed in a separate 100 mL volumetric flask and diluted to the mark with 99.8% methanol to create solution **1B**. 10 mL of the biphenyl solution was transferred to a 100 mL volumetric flask via a volumetric pipet and the flask was diluted to the mark with methanol to create a dilute biphenyl solution to create solution **1C**. Volumetric pipets and volumetric flasks were used to create solution **1D** from 25 mL of **1A** and 10 mL of **1B** diluted to 50 mL, **1E** with 10 mL of **1A** and 5 mL of **1B** diluted to 25 mL, **1F** with 15 mL of **1A** and 10 mL of **1B** diluted to 50 mL, **1G** with 10 mL of **1A** and 10 mL of **1B** diluted to 50 mL, **1H** with 5 mL of **1A** and 10 mL of **1B** diluted to 50 mL, **1I** with 30 mL of **1C** and 10 mL of **1B** diluted to 50 mL, **1J** with 10 mL of **1C** and 5 mL of **1B** diluted to 25 mL, **1K** with 5 mL of **1C** and 5 mL of **1B** diluted to 25 mL, and **1L** with 10 mL of **1C** and 20 mL of **1B** diluted to 100 mL. All solutions (**1A-1L**) were mixed by inverting 5 times. Three GCMS samples were taken for solutions **1D- 1L** using the NiTol-2 GC-MS method. Multipoint external standard calibration curves were prepared for both total ion count (TIC) and single ion 154.00 mass per charge (SI 154) and multipoint internal standard calibration curves were prepared for TIC biphenyl over TIC ethylbenzene and for SI 154 biphenyl over SI 106 ethylbenzene. The mass spectrometer was recalibrated, and the GCMS spectra were required for solution **1D-1L**. New multipoint external standard calibration curves were prepared for both total ion count (TIC) and single ion 154.00 mass per charge (SI 154) and multipoint internal standard calibration curves were prepared for TIC biphenyl over TIC ethylbenzene and for SI 154 biphenyl over SI 106 ethylbenzene.

Toluene Calibration Curve Preparation:

0.1662 g toluene was massed in a 100 mL volumetric flask and diluted to the mark with 99.8% methanol to create solution **2A**. 0.1002 g of 99% ethylbenzene was massed in a separate 100 mL volumetric flask and diluted to the mark with 99.8% methanol to create solution **2B**. 10 mL of the toluene solution was transferred to a 100 mL volumetric flask via a volumetric pipet and the flask was diluted to the mark with methanol to create a dilute toluene solution to create solution **2C**. Volumetric pipets and volumetric flasks were used to create solution **2D** from 25 mL of **2A** and 10 mL of **2B** diluted to 50 mL, **2E** with 10 mL of **2A** and 5 mL of **2B** diluted to 25 mL, **2F** with 15 mL of **2A** and 10 mL of **2B** diluted to 50 mL, **2G** with 10 mL of **2A** and 10 mL of **2B** diluted to 50 mL, **2H** with 5 mL of **2A** and 10 mL of **2B** diluted to 50 mL, **2I** with 30 mL of **2C** and 10 mL of **2B** diluted to 50 mL, **2J** with 10 mL of **2C** and 5 mL of **2B** diluted to 25 mL, **2K** with 5 mL of **2C** and 5 mL of **2B** diluted to 25 mL, and **2L** with 10 mL of **2C** and 20 mL of **2B** diluted to 100 mL. All solutions (**2A-2L**) were mixed by inverting 5 times. Three GCMS

samples were taken for solutions **2D- 2L** using the NiTol-2 GC-MS method. Multipoint external standard calibration curves were prepared for both total ion count (TIC), single ion 91.00 mass per charge (SI 91), and single ion 65.00 mass per charge (SI 65). Multipoint internal standard calibration curves were prepared for TIC toluene over TIC ethylbenzene, for SI 91 toluene over SI 106 ethylbenzene, and for SI 65 toluene over SI 106 ethylbenzene.

Failed Competition Reactions:

Seven completive nitration reactions were attempted with only one being used for relative rate of reaction analysis. The other six reactions failed due to either toluene evaporation, lack of nitration, and/or inaccurate quantification. The previous competition reactions used the same procedure detailed in the experimental section above but with the following deviations.

First attempt: Sodium nitrate was used instead of nitric acid, though it resisted dissolving in 17.45 M acetic acid. Biphenyl was not dissolved before combination with the nitrate and sulfuric acid. A boiling water bath was used, and the reaction flask was not stoppered. Reaction quenched with diethyl ether, then sodium bicarbonate instead of quenching with both simultaneously. Pressure released after inverting solution during methanol dilution, but no salt was generated. Quantification assessed as a failure due to molar toluene loss vastly exceeding molar sodium nitrate utilized.

Second attempt: Sodium nitrate was used instead of nitric acid, though it resisted dissolving in 17.45 M acetic acid. A boiling water bath was used, and the reaction flask was not stoppered. Pressure released after inverting solution during methanol dilution, but no salt was generated. Quantification assessed as a failure due to molar toluene loss vastly exceeding molar sodium nitrate utilized.

Third attempt: Sodium nitrate was used instead of nitric acid, though it resisted dissolving in 17.45 M acetic acid. A hot water bath (initial temperature 58.9 degrees Celsius, final temperature 90.9 degrees Celsius) was used, and the reaction flask was stoppered, but the stopper was faulty. Pressure released after inverting solution during methanol dilution, but no salt was generated. Quantification assessed as a failure due to molar toluene loss vastly exceeding molar sodium nitrate utilized.

Fourth attempt: Sodium nitrate was used instead of nitric acid, though it resisted dissolving in 17.45 M acetic acid. The solution was refluxed using a heating mantel and a cold-water condenser. Extraction used hexane isomers instead of diethyl ether. Pressure released after inverting solution during methanol dilution and white salt generated. Quantification assessed as a failure due to quantification showing higher amount of molar toluene after reaction than added to the reaction flask.

Fifth attempt: Sodium nitrate was used instead of nitric acid, though it resisted dissolving in 17.45 M acetic acid. The solution was heated in 50 degrees Celsius water and stoppered and clamped. Extraction used hexane isomers instead of diethyl ether. Pressure released after inverting solution during methanol dilution and white salt generated. Quantification assessed as a failure due to quantification showing higher amount of molar toluene after reaction than added to the reaction flask and chromatogram showing no nitrated toluene or nitrated biphenyl products.

Sixth attempt: Sodium nitrate was used instead of nitric acid and acetic anhydride used instead of concentrated acetic acid. The solution was reacted at room temperature and stoppered and clamped. Extraction used hexane isomers instead of diethyl ether. Pressure released after inverting solution during methanol dilution and white salt generated. Quantification assessed as a failure due to the chromatogram showing no nitrated toluene or nitrated biphenyl products and the hexane phase diluted in methanol in the volumetric flask not being homogenous.

The fourth, fifth, sixth, and seventh (utilized) competition reactions were performed simultaneously on different stir plates and used the same solution of ethylbenzene as an internal standard. The inconsistency of detector response for ethylbenzene was assessed to be negligible (>3% relative standard deviation) for competitions that had homogenous sample dilution in volumetric flasks shown in Supplemental Table 1. The failure of the multipoint internal standard to assess reagent molar quantities after competition reactions appears to not be related to the ability of the detector to repeatably assess ethylbenzene detector response.

Assessment of Failures of Ethylbenzene as an Internal Standard:

Sample error preparation is the likely cause of trends observed in Tables 1 and 2; when preparing biphenyl, toluene, and ethylbenzene in volumetric flask for calibration curves, the volumetric flask was tared and solid biphenyl was tapped into the flask, toluene was added dropwise, while ethylbenzene was poured. Because a funnel was not used to pour the ethylbenzene, ethylbenzene may have spilled onto the sides of the flask and massed without presence in the final solution; this error would have been variable and explains why the ratio of ethylbenzene concentration to detector response is repeatably consistent for different analytes that used the same sample of massed ethylbenzene but variable for samples that utilized a different aliquot of massed ethylbenzene. If this conclusion is correct, future analysis should avoid using the ethylbenzene internal standard calibration curves unless the curves are re-assessed, massing by adding ethylbenzene dropwise.

However, sample preparation does not explain the decrease in ethylbenzene detector response over time. This is seen in supplemental Figure S4, which graphs the detector response for TIC of ethylbenzene on GC-MS when preparing the toluene calibration curves; the same concentration of ethylbenzene was prepared for each solution (**2D- 2L**) from the same source solution **2B**. However, a clear decline in detector response for ethylbenzene over time is observed, with the most likely explanation being evaporation loss. This is complicated, though, by the presence of the decline in the biphenyl calibration curves (Figure S5) appearing to be influenced by the calibration of the MS; the toluene curves were prepared three months after the MS was calibrated. A similar trend in declining detector response over time is not observed with either biphenyl (Figure S6) or toluene (Figure S7), though the trend is obscured by the purposefully varied concentrations of toluene and biphenyl to create linear correlations. Because toluene and biphenyl do not show significant evaporation over time, but ethylbenzene appears to and because the biphenyl standards were run in sequence from most concentrated to least concentrated, the slope of the biphenyl internal standard curves are partially dependent on the order the sequence was generated in, which may account for the discrepancies noted in Table 3, though this is questionable considering how dynamic the discrepancies are. A similar conclusion for the internal standard curves for toluene calibration can be drawn but complicated by the fact that the solution was not run from most concentrated to least concentrated due to mislabeling the volumetric flasks **2A** and **2C**; this is noted in Figure S6.

Reagents Utilized:

Sodium Bicarbonate Saturated

$PdCl_2$ in 5% HCl 1 mg/mL Pd

Toluene Fisher Science Education Reagent Grade Code: S25611A Lot# 9GDC18I19DRM UN1294

Ethyl Ether Anhydrous Fisher Chemical E138-500 CAS 60-29-7 $C_4H_{10}O$ F. W. 74.12 Expires 02/2021 Lot 195162 Certified ACS BHT Stabilized 0.05% ethanol 99.8% ether, <0.0001% carbonyl compounds, <0.01 ppm copper

Sodium Sulfate, minimum 99.0% SIGMA® S9627-500G Batch #083K0156 EC 231-820-9; WGK 1; CH-Gift 5;

Magnesium Sulfate Anhydrous

Sodium Nitrate

Sulfuric Acid 18 M

2-bromotoluene TGI >98.0% B0659 CAS 95-46-5 SG 1.42 Lot. DSKE-OQ

Acetic Acid, Glacial

Saturated NaCl (Brine)

Dichloromethane

Exp. 2 Bakelite Phenol CHM 3373

Potassium hydroxide 1M

Potassium Hydroxide Certified A.C.S. Pellets Fisher Scientific FL-06-0597 Potassium Hydroxide, Solid UN1813 86.6% KOH, 0.2% K_2CO_3, 0.019% Ammonium Hydroxide Precipitate, 0.002% Chloride, 0.0005% Iron, 0.0001% Nickel, 0.0005% Nitrogen compounds, 4.9ppm Phosphate, 0.02% Sodium, 0.0009% Sulfate, Heavy metals (as Ag) 0.0009%, LOT NO 037403 CAS 1310-58-3

Anisole

Acetic Anhydride

Biphenyl 99% Acros Organics Code 106252500 CAS: 92-52-4

4-Bromotoluene Acetic Anhydride

Aldrich Chemical Company, Inc. Ethylbenzene, 99% E1,250-8 IR and GC analyzed Lot No MB

Acetic Acid 17.45M

Acetic Anhydride

Fisher Chemical Nitric Acid Certified ACS Plus LOT 156829 UN 2031 CAS 7697-37-2 Specific gravity 1.42 Normality 15.8 68.0 to 70.0 w/w%

3-Bromotoluene TGI B0660 >98.0% SG 1.41 CAS 591-17-3

Alfa Aesar 2-Chlorotoluene, 98% B23596 LOT: 10194318 CAS 95-49-8

ACROS N-Bromosuccinimide, 99% 10745-1000 CAS# 128-08-5 LOT# B0120628

Fisher Scientific Hexanes HPLC Grade Also meets ACS Specification Packed under Nitrogen Submicron Filtered Sade-Cote®, 35 L-18412, UN1208 99.9% Hexanes, CAS 110-5403 Hexane (contains a mixture of isomers) LOT 137351

Alfo Aesar Methanol, ultrapure, HPLC Grade, 99.8+% Liquid 22909 4L Lot: P23C700 CAS 67-56-1 EINECS 200-659-6

Supplemental Data:

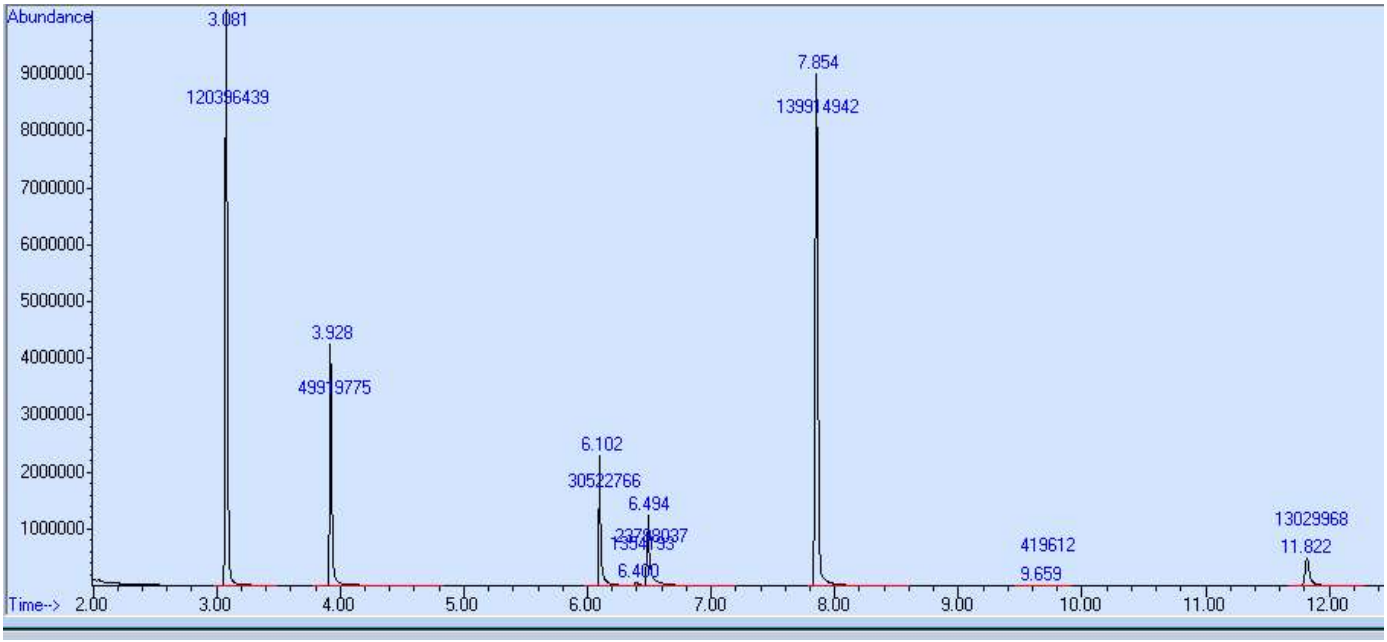

*Figure S1. Products of nitration of biphenyl and toluene with concentrated nitric acid in concentrated acetic acid extracted in diethyl ether and diluted in methanol (**VH-04-29-2021-4a**). Retention time (top number) and peak integration (bottom number) for each resolved peak shown; analytes analyzed are toluene (retention time 3.081 minutes) and biphenyl (retention time 7.854). An internal standard, ethylbenzene (retention time 3.928) was included but not used in calibration due to inconsistent concentration assessment contrasted to multipoint external standard calibration. Nitrated toluene products appear with retention times 6.102 minutes and 6.494 minutes and one of the nitrated biphenyl products appears at 11.822 minutes.*

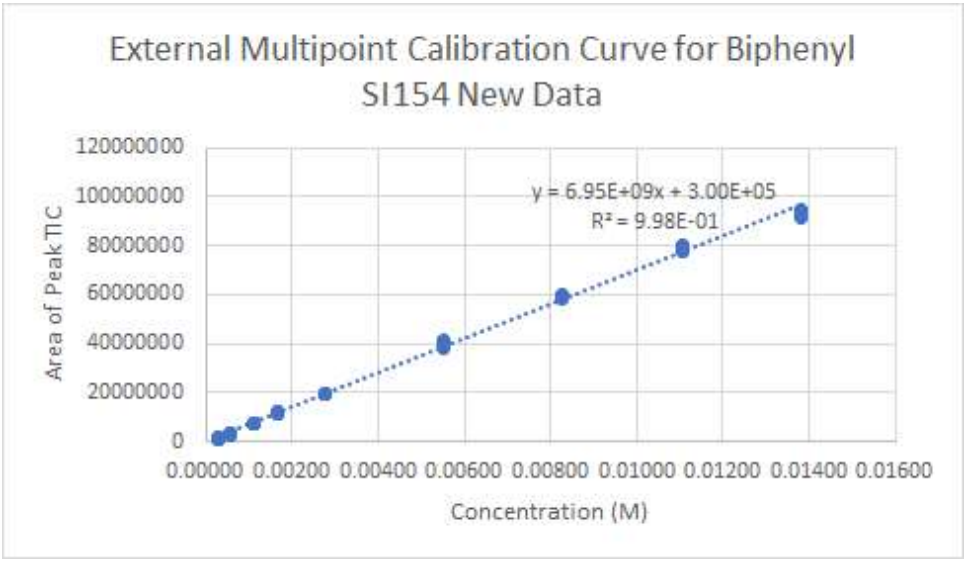

Figure S2. External Multipoint Calibration Curve for biphenyl used to assess biphenyl concentration after competitive nitration with toluene.

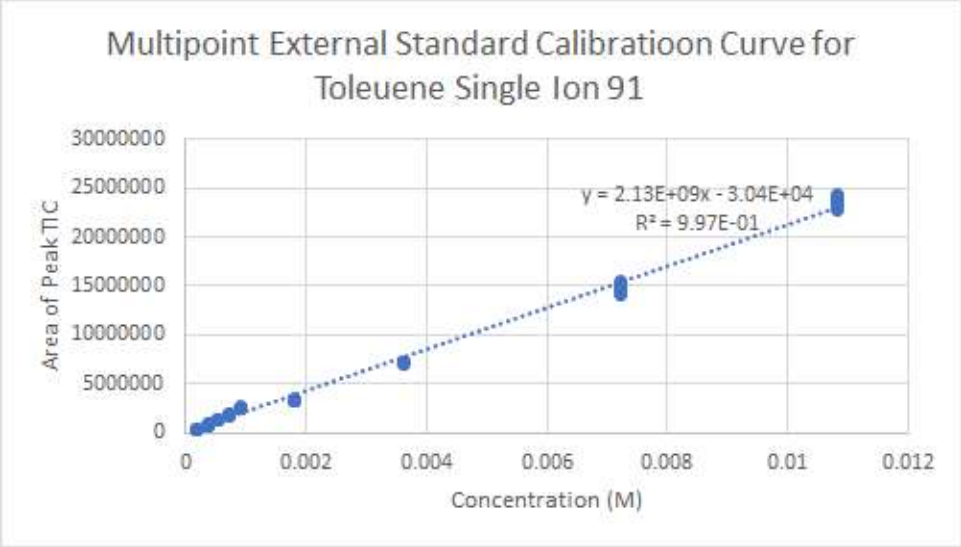

Figure S3. External Multipoint Calibration Curve for toluene used to assess biphenyl concentration after competitive nitration with toluene.

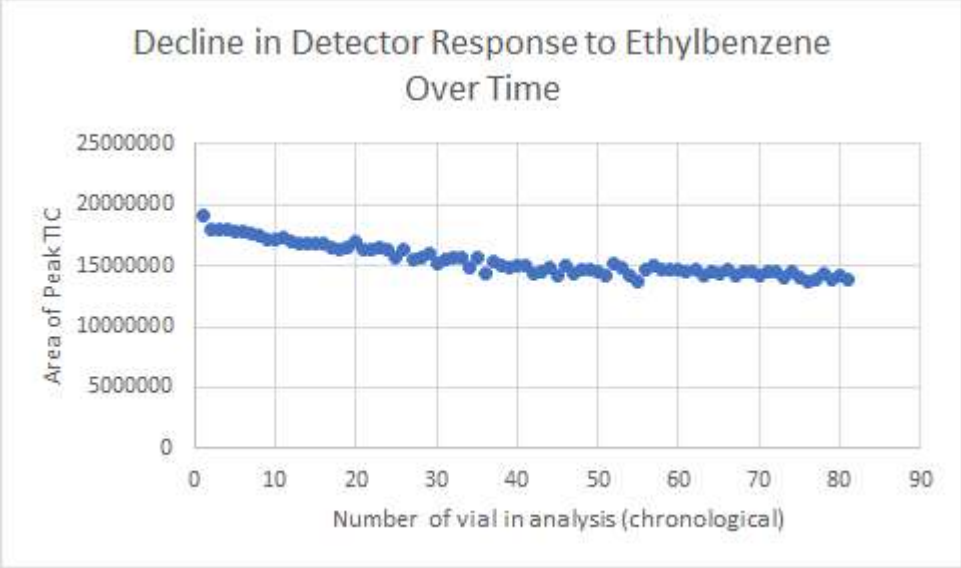

Figure S4. Graphical depiction of detector response of ethylbenzene—measured by TIC area of peak integration—against the position in the sequence the vials were run on the autosampler while preparing the toluene calibration curves. Each run lasted 22.6 minutes and 81 samples were analyzed. The decline in detector response over time is believed to be caused by evaporation of the ethylbenzene from the GCMS vials while the solutions wait for analysis.

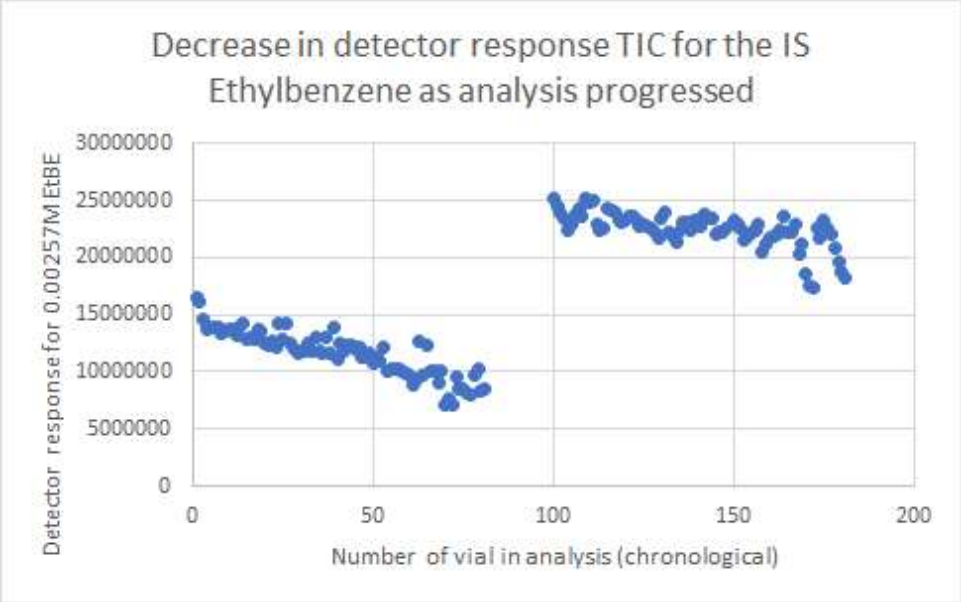

Figure S5. Graphical depiction of detector response of ethylbenzene—measured by TIC area of peak integration—against the position in the sequence the vials were run on the autosampler while preparing the biphenyl calibration curves. The samples were originally analyzed before the MS was recalibrated (left most 81 samples) and were ran again (right most 81 samples) after the MS was recalibrated, 6 days after solution preparation. Each run lasted 22.6 minutes. The decline in detector response over time is believed to be caused by evaporation of the ethylbenzene from the GCMS vials while the solutions wait for analysis; however, recalibration of the MS reduced the severity of this decline and nearly doubled detector response for ethylbenzene despite the solutions sitting (and presumably evaporation) on the autosampler for 6 days.

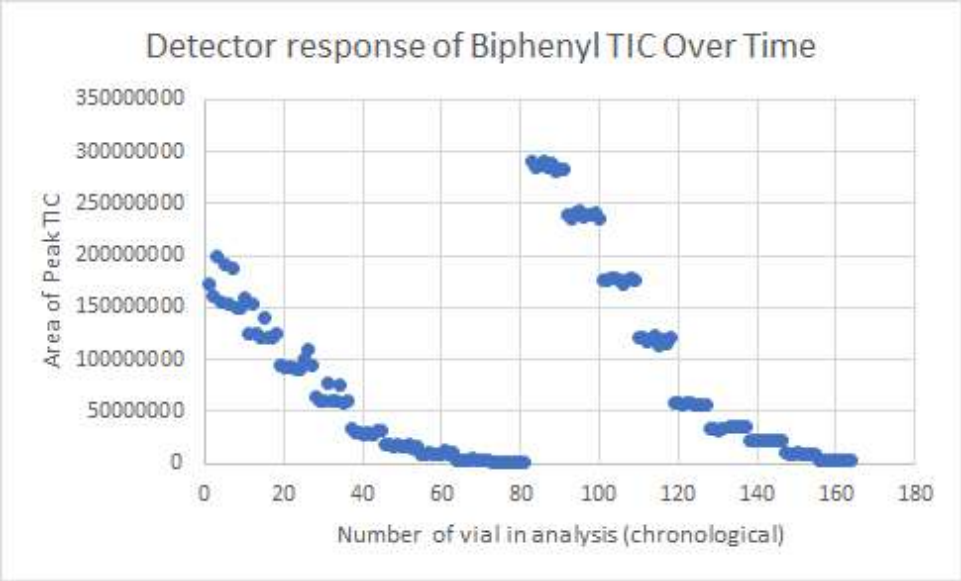

Figure S6. Graphical depiction of detector response of biphenyl– measured by TIC area of peak integration—against the position in the sequence the vials were run on the autosampler while preparing the biphenyl calibration curves. The samples were originally analyzed before the MS was recalibrated (left most 81 samples) and were ran again (right most 81 samples) after the MS was recalibrated, 6 days after solution preparation. Each run lasted 22.6 minutes. Slight decreases in biphenyl detector response over time may be present after the MS was recalibrated, though certain concentrations also show slight increases in biphenyl detector response over time, so these trends may just be caused by indeterminacy.

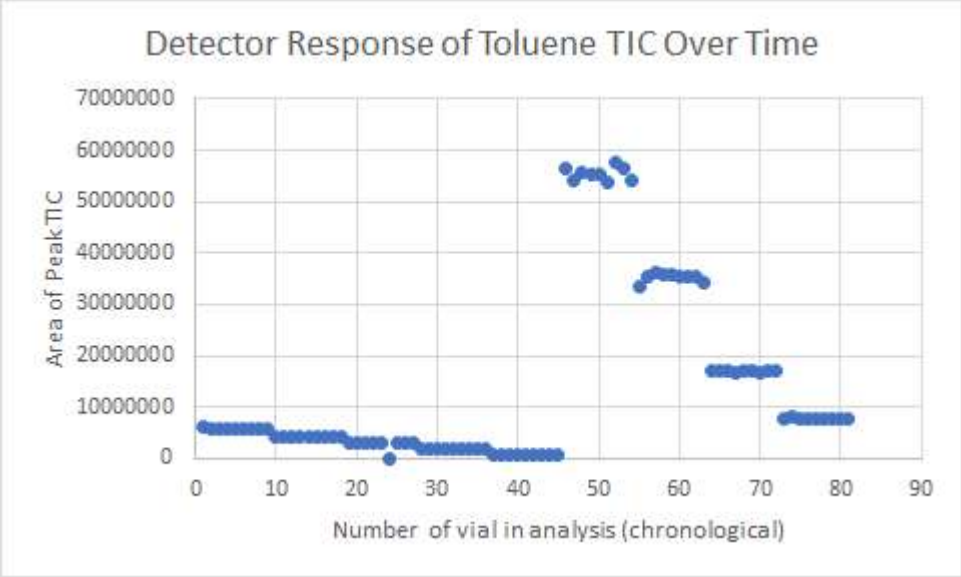

Figure S7. Graphical depiction of detector response of toluene—measured by TIC area of peak integration—against the position in the sequence the vials were run on the autosampler while preparing the toluene calibration curves. Each run lasted 22.6 minutes and 81 samples were analyzed. The solution was run decreasing from most concentrated to least concentrated though the labels on the solutions used to prepare the four most concentrated solutions was confused with the solution used to prepare the five least concentrated solutions; this is clearly visible on the graph between the first 45 samples (left) analyzed and the remaining 36 samples (right). No clear trend is observed for the decrease in detector response over time.